The axolotl limb regeneration mutant strain, short toes (s/s), can regenerate spinal cord and tail, but not limbs. These properties are very interesting for limb regeneration studies. Recently, we reviewed this mutant (Dev. Growth Differ. (2007) 49, 469–478.), and here we will report a more extensive molecular information. In the short toes limb, some genes related to pattern formation in development and regeneration (shh, FGF-8, FGF-10) are expressed at the same level as in normal limb. On the other hand, the stem cell marker genes Msi(musashi)-1 and Msi-2, MyHC (myosin heavy chain)-1, MyHC-2b, MyHC-4 and Pax-7 are down-regulated in s/s limb. In particular, all three MyHC genes and Pax-7 are highly expressed in the normal limb, but almost lost in s/s limb. MyHC genes are one of the main components of skeletal muscle, and Pax-7is the skeletal muscle satellite cell marker. These results suggest s/s limb may have a normal potential of pattern formation, but not have stem cells and/or muscle satellite cells. When a +/+ limb blastema was grafted on a s/s host limb, the +/+ blastema developed through digit formation stage, but with abnormalities. This suggests that there is an effect of the s/shost, perhaps through growth factor production or innervation defects. These studies suggest that patterning potential may be sustained in s/s but some step of regeneration, probably related in stem cell and/or muscle satellite cell, is dysfunctional. Funded by the William M. Keck Foundation.

doi:10.1016/j.ydbio.2008.05.326

Program/Abstract # 307
Expression of matrix metalloproteinases (MMPs) during axolotl limb regeneration

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Axolotl (*Ambystoma mexicanum*) is one of the rare animals which can perfectly regenerate their organs and tissues, such as limbs, after amputation. During limb regeneration, the remodeling of the extracellular matrix (ECM) and the activation of various growth factors play critical roles in regenerating correct limbs. Matrix metalloproteinases (MMPs), a group of zinc-dependent endopeptidases, are able to cleave the major components of the ECM which result in ECM remodeling, and activate various growth factors. The primary goal of this study is to examine the expression of MMPs in the axolotl limb blastema from different regeneration stages. Various MMP protein arrays (RayBiotech,Inc., Norcross, GA) were able to detect multiple MMPs including MMP-1, -2, -3, -8, -9, -10, and -13, as well as their intrinsic inhibitors, TIMP-1, TIMP-2 and TIMP-3, in the blastema collected 1, 4, and 7days after amputation. Zymography demonstrated that after amputation that several MMPs with migrating of 92 kDa, 72 kDa, 64 kDa and 54 kDa, have increased activities. These data suggested that MMPs, especially 92 kDa, 72 kDa, 64 kDa and 54 kDa, might be responsible for the ECM remodeling and growth factor activation that occurs during the axolotl limb regeneration after amputation.

doi:10.1016/j.ydbio.2008.05.327

Program/Abstract # 308
Functional genomics of planarian regeneration

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Regenerative wound healing in adult organisms has been studied for over 100 years. It is a remarkable ability with conservation throughout the evolutionary tree; however, the ability varies from branch to branch. We are using functional genomics to identify and characterize genes shared by regenerative organisms. We hypothesize that certain genes are conserved in animals capable of regenerative wound healing as adults. Planarians are well known for their regenerative capabilities. An expressed sequence tag (EST) library derived from the planarian *Schmidtea mediterranea* was used for comparative analyses; 393 unique genes are conserved between planarians and animals that can regenerate as adults. All are conserved in vertebrates and ~50% are predicted or novel proteins. In silico characterization of the 393 ESTs using SignalP, Gene Ontology and Clusters of Orthologous Groups suggest ~15% are secretory proteins and ~30% are involved in signal transduction. Expression patterns of the ESTs were determined in whole planarians by *in situ* hybridization. RNA interference is being used to knock down individual expression of the ESTs, and regeneration efficacy after bisection will be assessed. Effects of RNAi are determined visually and by immunohistochemistry. Preliminary data indicate ~30% of the knockdowns elicit phenotypes, including: defects in nervous system and body patterning; delays and failures of photoreceptor regeneration; blastema regression; lesioning; and death. Integrating *in silico* analyses with characterization of gene expression patterns and RNAi phenotypes will provide a systems-level analysis of the conserved genes' functions in planarians.

doi:10.1016/j.ydbio.2008.05.328

Program/Abstract # 309
Intestinal renewal and regeneration in the planarian *Schmidtea mediterranea*

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In planarians, pluripotent somatic stem cells called neoblasts continuously replenish tissues during normal cellular turnover and regenerate missing tissues after injury. However, the dynamics of renewal and regeneration of planarian organ systems are not well characterized at the cellular or molecular level. Addressing these problems in the intestine of *Schmidtea mediterranea,* we conducted time course experiments using immunohistochemical markers, bromodeoxyuridine labeling, whole mount in situ hybridization, and fluorescent labeling of intestinal cells in living animals. Our observations indicate that as expected, intestinal epithelial cells do not actively cycle, but instead are derived from neoblasts in intact, uninjured animals. Intestinal regeneration proceeds by both significant remodeling of differentiated intestinal tissue (morphallaxis) as well as by differentiation of new intestinal epithelial cells post-injury (epimorphosis). Concurrently, we are conducting a monoclonal antibody screen to generate intestine-specific markers, as well as microarray-based analysis of gene expression in the intestine. Results of these ongoing efforts will be presented. (This work was supported by NIH-NICHD R01 HD043403 to PAN and by NIH-NIDDK F32 DK077469 to DJF.)

doi:10.1016/j.ydbio.2008.05.329